Behavior as a Tool to Assess the Effects of Developmental Methylmercury Exposure in Zebrafish

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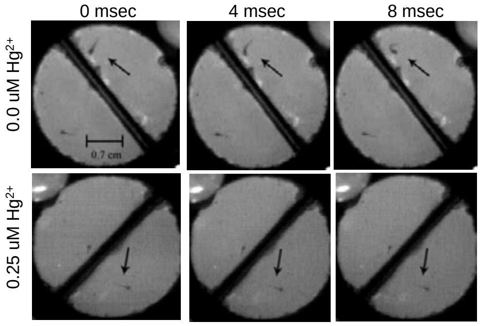
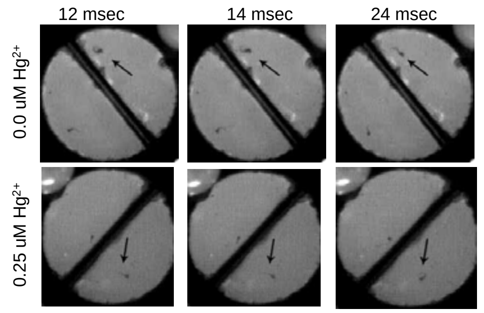
Introduction: Ontogenetic critical periods exist in many vertebrate species during which sensitivities to environmental chemical and physical stress will vary. In fishes, toxic exposure at early stages of development are more effective at producing behavioral deficits in locomotor function, feeding behavior and social interactions. Use of behavioral teratology techniques is beneficial in identifying the underlying, age-specific, neural mechanisms affected by toxic exposure. The current study presents observations on 1) a high-performance larval behavior utilizing high-speed videography to demonstrate alterations in the escape kinematics and 2) learning behavior after embryonic exposure to sublethal concentrations of mercury (Hg) as a first step toward evaluating the intergenerational effects of adult maternal dietary exposure to mercury compounds, a key issue in the environmental health of both humans and wildlife.

This study used zebrafish due to ease of egg production in the laboratory, short hatching time (3 days), lower cost than warm-blooded models, and its use as a sentinel for aquatic pollution. This study was guided by the hypothesis that prehatch Hg exposure during specific periods of development will specifically affect the function of certain sensory and/or neuromuscular neurons, and higher learning centers. These changes could result in short- and long-term changes in escape responses to specific environmental stimuli, as well as ability to learn specific tasks.

Methods and Results: Zebrafish embryos (2 hours post fertilization; hpf) were exposed to 0-0.3 uM Hg^{2+} until 24 hpf. Tests for response to a low frequency, hydrostatic wave created by a hammer hitting a pin near the fish chamber were conducted at 7 days post fertilization (dpf). Behavior was analyzed for latency of reaction, maximum velocity, and duration of behavior.

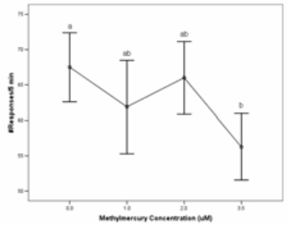


Control larvae begin to react to the vibrations in the water as Early as 4 msec and have completely turned within 8 msec.



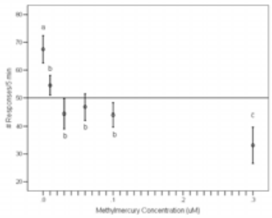
Even those control larvae that show a greater delay in reaction time have completed there response before the larvae from treated embryos have reached maximum velocity at 24 msec.

Responses to a visual stimulus, a rotating black bar under very low illumination (~60 uW/m^2) also display a dose-dependent pattern. Initially, offspring of females fed varying levels of Hg-contaminated walleye, were evaluated at 4 months of age (A.). Parallel studies involving waterborne exposures to methylmercury to embryos 0-24 hpf were also conducted (B.).

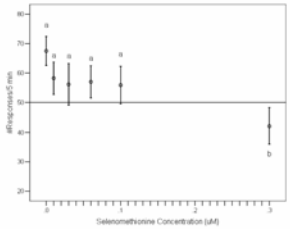


A.

B.

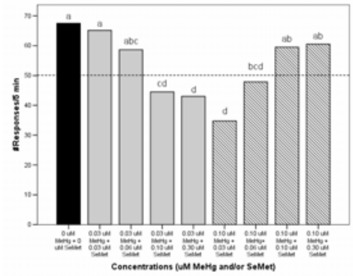


Clearly, even low levels of waterborne methylmercury induce visual deficits in terms of response rate to a directional stimulus. Yet, offspring from females fed a methylmercury-contaminated diet did not show visual deficits unless females were fed the higher concentration diet. Why? It is possible that walleye contain nutrients that counter the effects of Hg. To assess that possibility embryos were exposed to various combinations of methylmercury and selenomethionine (C, D).



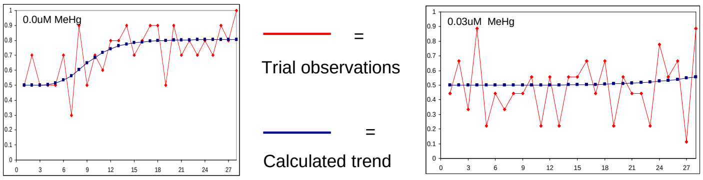
C.

D.



Selenomethionine is found in a variety of foods, including walleye. Alone, this form of selenium has no negative effect on visual response rate, except at high concentrations (C.). Embryos co-exposed at ratios of approx. 1Se:1Hg produced adults with little or no visual deficits (D.).

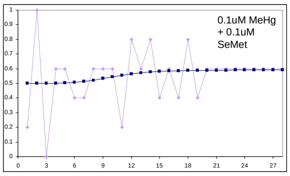
E.



F.

Using a spatial alternation task paradigm in which individual fish at 4 months, exposed as embryos only to methylmercury and/or selenomethionine, must learn a pattern of food delivery, Control fish (E.) are able to learn the task within approx. 12-14 trials (probability >0.75). Fish expose as embryos to even low concentrations of methylmercury (F.) have greater difficulty in learning the pattern, although it is possible, in examining the later trials, that a delay in learning is a more appropriate conclusion. While not shown, higher exposure concentrations produced a more flat line response, i.e., response to feeding cues was no different from random (probability = 0.5).

G.



We are also interested in determining if selenium co-exposure would, as with visual responses, reduce the effect of mercury toxicity. To date, data are inconclusive (G.) and additional testing is required. It is interesting that there is a range of Se:Hg ratios that meliorate visual toxicity (1-3:1). It is possible that learning, a more complex process, requires a Se:Hg ratio more on the high end of that range.

Conclusions: Due to similarities to human postnatal/adult behavioral deficits, zebrafish serve as a biomedical model to assess short- and long-term behavioral abnormalities from developmental MeHg exposure. Since behavioral effects of developmental MeHg exposure were reduced by co-exposure to SeMet, such dietary supplements may have clinical importance to human health.

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